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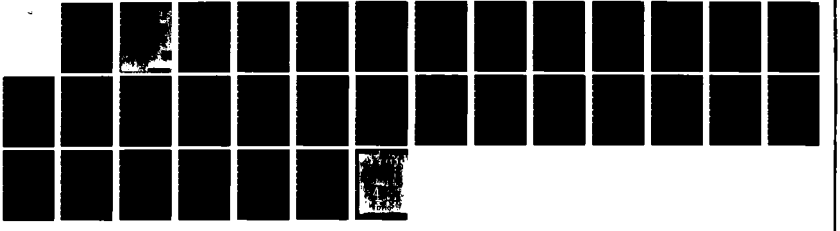

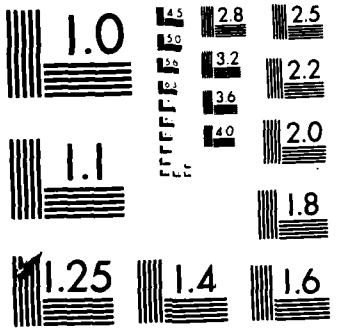

2nd Annual Report on

EFFECT OF ANTIPARASITE CHEMOTHERAPEUTIC AGENTS ON IMMUNE REACTIONS

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EFFECT OF ANTIPARASITE CHEMOTHERAPEUTIC AGENTS ON IMMUNE REACTIONS

Abdul Ghaffar, PhD

August 1980

Supported by the

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SUMMARY

Immunotoxic effects of four agents, WR 171669, WR 142490, WR 172435 and WR 180409, were studied.

WR 171669, at maximal sublethal dose, caused a slight increase in the antibody plaque-forming cell (PFC) response, and marginal suppression in the delayed hypersensitivity (DH) reaction and *in vivo* phagocytic function.

WR 142490 caused slight reduction in the splenic cell content at the higher dose given before or after the antigen. It did not significantly affect antibody PFC responses at any dosage or timing. The DH reaction was suppressed by this agent given before or after the antigen but the statistical significance was demonstrable only in the group receiving a high dose of the drug before the antigen. WR 142490 injected at 10 mg/kg but not 40 mg/kg caused a significant augmentation of the phagocytic function.

WR 172435 caused a significant reduction in IgG response in only one instance: in mice treated with 10 mg/kg of the drug one day after antigen. Since this reduction was of a very modest magnitude and a higher dose of the drug failed to exert a similar effect, the biological significance of this effect is questionable. No statistically significant change in DH reaction was noted in mice treated with this agent; however, it caused significant augmentation of RE function. The augmentation in K value was significant in mice receiving both the high and low doses of the drug but the increase in α value was significant only in mice receiving the higher dose of the drug.

WR 180409, at the higher dose, caused a small but significant reduction in the cellularity of the spleen. Given at the higher dose before antigen it caused significant suppression of the IgG response but when given after the antigen it caused a slight augmentation. DH reactions were not at all altered by this drug. Only the lower dose of this agent caused slight augmentation of RE function, as reflected by the K value but not the α value.

In conclusion, none of these drugs had a dramatic effect on any of the immune parameters measured.

A-1

METHODOLOGY

Mice. Male Balb/c mice weighing 20-25 grams were used in all experiments. Animals were maintained according to the "Guide for the Care and Use of Laboratory Animals" (1972) prepared by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council (DHEW Publ. No. (NIH) 73-23).

Antigen and Immunization. Sheep erythrocytes (SRBC) suspended in normal saline were used for immunization. Mice received 0.2 ml of 2.5% (1×10^8) SRBC by the i.p. route for studies involving humoral response, whereas 0.1 ml of 0.25% suspension was injected s.c. on the dorsal side of the neck for immunization for the DH reaction. The challenge for elicitation of the DH reaction was given as 0.02 ml of 25% suspension i.d. in the right ear.

Humoral Responses. Humoral IgM and IgG responses against SRBC were measured by assaying individual spleens for the hemolytic PFC contents by the method described by Cunningham and Szenberg (1).

Five days following i.p. immunization of mice with SRBC, spleens were excised and gently disrupted by means of a glass/Teflon tissue homogenizer in Hank's balanced salt solution containing 0.1% bovine serum albumin (BSA-HBSS). The cells were washed once and resuspended in 4 ml of the same medium. The tubes were allowed to stand for 60 sec to allow clumps and tissue debris to settle. A 1 ml aliquot of singly dispersed cells was removed from the top of the suspension and transferred to another tube. The number of nucleated cells per ml of this suspension was enumerated and adjusted to a concentration of

2-3 x 10^6 nucleated cells per ml. One volume (0.1 ml) of this suspension was mixed with one volume of 10% SRBC suspension, one volume of medium and one volume of 1:2 dilution of complement. Aliquots (0.1 ml) of the mixture were placed in chambers prepared by assembling two 3" x 1" microscopic slides by means of a 5 mm wide double-faced adhesive tape. The chambers were sealed along the edges with molten wax and incubated at 37°C in a humidified incubator for 40 min after which the number of plaques was enumerated.

The above technique allowed the assay of the IgM PFC contents of the splenic suspension. For the detection of IgG PFC the splenic cell suspension was mixed with equal volumes of 10% SRBC, rabbit anti-mouse IgG serum at an optimal dilution (previously determined) and complement. The mixture was placed in chambers and incubated as above. Plaques observed in these chambers were a mixture of IgM and IgG, and the latter were derived by the deduction of the IgM PFC calculated from plaques observed in chambers containing no antiserum against mouse IgG (2). From the number of plaques per chamber was calculated the number of PFC per 10^6 nucleated splenocytes and also PFC per spleen.

Delayed Hypersensitivity Reaction. Mice were injected s.c. with 5 x 10^6 SRBC in 0.1 ml volume on the dorsal side of the neck. Six days later they were challenged with 1 x 10^8 SRBC in 20 µl volume i.d. in the pinnea of the ear; 24 hr later 2 µCi of ^{125}IUdR in 0.2 ml volume was injected i.v. via the tail vein. Both ears were excised 18 hr later at the base of the pinnea and the radioactivity incorporated in each ear was measured in a γ-scintillation

counter. The ratio of radioactivity incorporated in injected and non-injected ears was calculated and was taken as an index of the DH reaction. This method has been reported as a reliable procedure for assaying the DH reaction to a number of agents (3) and we have confirmed the validity of the technique in our own laboratory.

Phagocytic Function of the Reticuloendothelial System (RES). Clearance of SRBC labeled with ^{51}Cr (chromoium isotope) was used as a criteria for the RES function (4,5). SRBC were washed three times in saline and to 1 ml of packed SRBC was added 100 μCi of ^{51}Cr (but always less than 5 μg Na_2CrO_4). The cells were mixed and incubated for 30 min at room temperature and then washed three times in saline. Washed cells were suspended at a final concentration of 10% (v/v) in saline and 0.2 ml of this suspension (4×10^8 SRBC) was injected i.v. via the tail vein and 20 μl of blood was collected immediately (zero time sample) from the retroorbital sinus using a heparinized capillary (hematocrit) tube. The same volumes of samples were collected similarly at 2, 5, 10 and 15 min intervals from the time of original bleeding. All samples were placed in test tubes and the radioactivity was counted in a gamma scintillation spectrometer. The radioactivity in the sample represented the relative concentration of SRBC in circulation at a particular time. The cpm were converted to log and plotted against time, and the slope of the best fitting straight line for the plot was calculated; it represented the phagocytic index K. Since the concentration of particles in circulation and hence the K value is dependent on the total body weight of the mouse, its liver and spleen weight, a corrected phagocytic index, α, was calculated as follows:

$$\alpha = \frac{w}{l + s} \sqrt[3]{K}$$

where w is whole body weight, l is liver weight and s is spleen weight.

In one earlier experiment, colloidal carbon (Pellikan India ink) was used as the particulate material instead of SRBC. In this experiment (see results on WR 171669) the concentration of the particulate material in the blood was measured spectrophotometrically and the K and α values were calculated from the slope of the curve obtained by plotting $\log_{10}$ optical density of samples measured at 800 nm against time (6).

Localization of Particulate Material in Liver and Spleen. Since the particulate material from circulation is cleared mostly by entrapment in the liver and spleen (4), any alteration in the organ weight or functional activity of the Kupffer cells or dendritic splenic macrophages would affect the clearance rate. Use of ^{51}Cr-labeled SRBC enabled us to determine their organ localization. Spleen and liver were removed intact and weighed. The radioactivity associated with each organ (proportionate with their SRBC content) was measured in a gamma counter and the radioactivity was calculated per gram of the organ weight.

Presentation of Data. Numbers of spleen cells have been presented as geometric means. PFC per 10^6 splenocytes and per spleen have been recorded as $\log_{10}$ mean together with one standard error. Anti-log of $\log_{10}$ mean PFC are recorded in parentheses. DH reaction has been reported as the mean of relative ear reaction together with the standard error of the mean. The relative ear reaction was calculated as follows:

$$\frac{^{125}\text{IUdR incorporation (cpm) in challenged ear}}{^{125}\text{IUdR incorporation (cpm) in contralateral ear}}$$

RE function has been expressed as the arithmetic mean of phaogyctic index, 100 x K values or α values corrected for variations in the whole body weight and liver and spleen weight of individual mice within the limit of one standard error. Spleen and liver organ weight has been presented as the mean of the organ weight in mg per gram of body weight and the uptake of particulate material by these organs has been expressed as the mean of radioactive SRBC content (cpm) per gram organ weight ± one s.e.

Statistical significance of the data was evaluated by the standard two-tailed Student's t-test with correction for small groups and expressed as p values. P values greater than 0.05 were considered not significant.

Solvent and Dosage. WR 171669 (BB41223, AGC-W100.1, May 4, 1979) was suspended in DMSO:Tween-80-Saline (4:1:15). This solvent was not toxic and gave a colloidal suspension. The required dose of the drug was injected i.p. in 0.4 ml. In initial experiments, 2000, 1500 and 1200 mg/kg of the drug were used but, due to the high toxicity at these doses, immunological studies were conducted at doses of 1000 and 250 mg/kg drug as salt.

WR 142490 AS HCl (BH10371, AP-VIII-153, March 19, 1980) and WR 180409 AD H_3PO_4 (BE99420, AP-VIII-112, March 19, 1980) were dissolved in 10% ethanol in saline (by dissolving in pure ethanol first and then diluting to the exact concentration with saline) and the desired dose (40 mg/kg or 10 mg/kg) was injected in 0.2 ml volume. Control mice received 10% ethanol in saline.

WR 172435 AK CH_3SO_3H (BG33210, AP-VIII-123, March 19, 1980) was suspended in 10% DMSO in saline (drug dissolved in DMSO and then diluted with saline to the exact concentration). The desired dose (50 mg/kg or 10 mg/kg) was injected in 0.2 ml volume. Control animals received a 10% solution of DMSO. From several experiments it was observed that 10% DMSO or 10% ethanol did not significantly alter immune responses of mice.

RESULTS

Humoral Responses. Humoral immune response as measured by the PFC content of spleens was slightly augmented by WR 171669 at the higher dose injected one day before the antigen (Table 1). This augmentation was statistically significant for IgM PFC per 10^6 splenocytes ($p < 0.05$), IgG PFC per 10^6 splenocytes ($p < 0.05$) and IgG PFC per spleen ($p < 0.005$). The drug injected after the antigen at the lower dose (250 mg/kg) also caused significant augmentation of IgG PFC per 10^6 splenocytes ($p < 0.005$). At the higher dose given after antigen this agent caused a significant reduction in the total IgM PFC content of the spleen. This reduction could be accounted for mostly by the reduction in the splenic cellularity (Table 1).

WR 142490 did not cause any significant changes in the PFC response of mice receiving either of the two doses of the drug, although the splenic cellularity of the mice injected with 40 mg/kg of the drug six days previously was slightly but significantly ($p < 0.05$) reduced (Table 2).

WR 172435 at the lower dose (10 mg/kg) caused a significant reduction in IgM per 10^6 splenocyte response when injected one day before SRBC ($p < 0.01$) and IgG per 10^6 splenocyte ($p < 0.02$) response when injected one day after the antigen (Table 3). Other than these, no significant changes in humoral responses were caused by this agent. The biological significance of the above mentioned statistically significant alterations could be questioned because of the magnitude of the changes and the failure of the higher dose to cause such an effect. Furthermore, the changes were significant only at the PFC per 10^6

level and not when total PFC contents of the spleen were considered.

WR 180409 at the higher dose injected before the antigen caused a significant ($p < 0.05$) reduction in the IgG per 10^6 splenocytes, IgM per 10^6 splenocytes and total splenic IgG responses (Table 4). Given after the antigen this agent at the lower dose caused a significant augmentation of the IgM per 10^6 splenocyte ($p < 0.05$) and total IgM PFC per spleen ($p < 0.02$) responses. The higher dose of WR 180409 injected before or after antigen also caused a significant reduction in the cellularity of spleen ($p < 0.025$).

DH Reactions. A significant ($p < 0.02$) reduction in the DH reaction was caused by the lower dose of WR 171669 injected one day before the antigen (Table 5). The higher dose given before or after the antigen also caused a depression in the DH reaction, although these changes were statistically not significant ($p > 0.05$).

WR 142490 also caused a statistically significant ($p < 0.025$) suppression when injected at 40 mg/kg one day after the antigen (Table 6).

None of the changes in the DH reaction of mice injected with WR 172435 (Table 7) or WR 180409 (Table 8) were statistically significant.

RE Function. The effect of WR 171669 was assessed by measuring the clearance rate of colloidal carbon from the circulation of mice treated with the drug or the solvent. From Table 9 it would appear that the higher dose of WR 171669 considerably impaired the RE function of mice and this impairment was significantly noticeable with both K values ($p < 0.001$) and α values ($p < 0.01$).

Colloidal carbon was replaced by radiolabeled SRBC for the RE function studies with WR 142490, WR 172435 and WR 180409. This was found necessary because of the difficulty in obtaining standardized colloidal carbon suitable for scientific investigations and because of toxicity of India ink (a source of colloidal carbon) in mice due to the presence of shellac (7). ^{51}Cr-labeled SRBC have previously been used as a particulate material for the study of the RE function (4,5).

The lower dose of WR 142490 significantly augmented both K ($p < 0.001$) and α ($p < 0.02$) values of phagocytic indices. The higher dose, however, failed to significantly influence either (Table 10). WR 172435 also significantly augmented the K value of the phagocytic index at both doses ($p < 0.005$ for 10 mg/kg, $p < 0.001$ for 50 mg/kg). However, only the higher dose of this agent caused a significant ($p < 0.025$) increase in the α value (Table 11). The higher dose (40 mg/kg) of WR 180409 did not have any significant effect on K or α values; however, it elevated the K value in mice injected with 10 mg/kg (Table 12).

Splenic and Hepatic Changes. Data on the effects of WR 142490, WR 172435 and WR 180409 on spleen and liver weight changes and the localization of ^{51}Cr-SRBC in these organs have been summarized in Tables 13-15. WR 142490 did not have any significant effect on the spleen or liver weights; however, it caused a slight but statistically significant ($p < 0.01$) drop in the splenic uptake of SRBC at the lower dose of 10 mg/kg (Table 13). WR 172435 had no significant effect on either splenic or liver weight or SRBC uptake at either the

higher or the lower dose (Table 14). WR 180409, at the lower dose, caused a significant increase in both splenic and hepatic weight; it also caused a reduction in the splenic uptake of SRBC (Table 15).

COMMENTS

None of the four agents tested had a profound effect on any of the immunological functions measured. Moderate to slight suppressive and sometimes augmentative effects exerted by certain agents, although statistically significant, may not have a biological significance. Most chemotherapeutic agents which expose the recipient host to infections are extremely suppressive at doses far below their limits of lethality. For example, cyclophosphamide well below the lethal dose causes over 90% depression in the response of mice to SRBC (8, and personal observations). Similar effects of a number of other alkylating agents have been noticed (9, and personal observations). Similarly, corticosteroids inhibit phagocytic and other functions of macrophages and T cells at doses well below lethality (10). Agents studied and results reported here have employed doses of drugs reaching lethal doses and their immunotoxic effects have been minimal. It was encouraging to note, from the point of their therapeutic potential, that three of four agents, viz., WR 142490, WR 172435 and WR 180409, potentiated the phagocytic function at one dose or the other and none suppressed this function. In view of the importance of phagocytic cells as the first stage of host defense against infection, their resistance to these antiparasitic agents is of considerable significance and their augmented function following drug treatment may be of some merit. Indeed, the real therapeutic value of these agents will depend on their antiparasitic effects.

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TABLE 1

Effect of WR 171669 on anti-SRBC PFC response of mice[a]

Drug Treatment		Cells/Spleen	PFC per[b] 10^6		PFC per[b] Spleen	
Time	Dose	x 10^{-6}	IgM	IgG	IgM	IgG
	Solvent	113.7	2.923 ± 0.069 (838)	2.769 ± 0.063 (587)	4.992 ± 0.061 (98,090)	4.838 ± 0.055 (68,856)
Pre Ag	250 mg/kg	150.9[c]	2.839 ± 0.066 (690)	2.901 ± 0.085 (796)	5.016 ± 0.063 (103,705)	5.078 ± 0.091 (119,738)
	1000 mg/kg	100.0	3.102 ± 0.036[c] (1,264)	3.110 ± 0.063[c] (1,287)	5.098 ± 0.045 (125,240)	5.109 ± 0.076[c] (128,446)
	Solvent	184.4	2.792 ± 0.052 (620)	2.773 ± 0.084 (593)	5.058 ± 0.060 (114,294)	5.039 ± 0.094 (109,369)
Post Ag	250 mg/kg	171.3	2.885 ± 0.029 (768)	3.034 ± 0.071[c] (1,083)	5.117 ± 0.033 (130,769)	5.269 ± 0.073 (185,842)
	1000 mg/kg	124.4[d]	2.729 ± 0.043 (538)	2.776 ± 0.068 (597)	4.822 ± 0.035[d] (66,436)	4.867 ± 0.086 (73,578)

[a] The drug was injected i.p. 1 day before or 1 day after 1 x 10^8 SRBC injected i.p. and PFC assays performed 5 days later.

[b] Log_{10} mean ± 1 s.e. from groups containing a minimum of 11 mice. Numbers in parentheses are geometric means for each group. Data pooled from 2 experiments.

[c] Statistically significant ($p < 0.05$ but > 0.01 for cells/spleen, IgM/10^6 and IgG/spleen, and $p < 0.005$ but > 0.001 for IgG/10^6.

[d] Statistically significant ($p < 0.005$ but > 0.001) reduction.

TABLE 2

Effect of WR 142490 on anti-SRBC PFC response of mice[a]

Drug Treatment		Cells/Spleen	PFC per[b] 10^6		PFC per[b] Spleen	
Time	Dose	$\times 10^{-6}$	IgM	IgG	IgM	IgG
	Solvent	204.7	3.110 ± 0.048 (1,289)	3.200 ± 0.114 (1,584)	5.417 ± 0.059 (261,196)	5.447 ± 0.134 (279,971)
Pre Ag	10 mg/kg	172.2	3.168 ± 0.059 (1,472)	2.999 ± 0.053 (997)	5.389 ± 0.038 (244,855)	5.123 ± 0.034 (132,715)
	40 mg/kg	160.1[c]	3.211 ± 0.056 (1,624)	3.163 ± 0.077 (1,457)	5.415 ± 0.058 (260,200)	5.369 ± 0.075 (233,859)
	Solvent	187.8	3.253 ± 0.024 (1,789)	3.420 ± 0.021 (2,631)	5.525 ± 0.024 (335,226)	5.655 ± 0.029 (452,171)
Post Ag	10 mg/kg	202.1	3.204 ± 0.063 (1,598)	3.351 ± 0.065 (2,241)	5.499 ± 0.059 (315,678)	5.631 ± 0.075 (427,395)
	40 mg/kg	153.7	3.338 ± 0.047 (2,176)	3.422 ± 0.094 (2,642)	5.524 ± 0.072 (334,329)	5.518 ± 0.075 (329,447)

[a]The drug was injected i.p. 1 day before or 1 day after 1×10^8 SRBC injected i.p. and PFC assays performed 5 days later.

[b]Log_{10} ± 1 s.e. from groups containing 10 mice for IgM and 5 mice for IgG. Numbers in parentheses are geometric means for each group. IgM data pooled from 2 experiments.

[c]Statistically significant reduction ($p<0.01$ but >0.0025).

TABLE 3

Effect of WR 172435 on anti-SRBC PFC response of mice[a]

Drug Treatment		Cells/Spleen	PFC per[b] 10^6		PFC per[b] Spleen	
Time	Dose	$\times 10^{-6}$	IgM	IgG	IgM	IgG
	Solvent	207.4	3.032 ± 0.051 (1,076)	2.867 ± 0.026 (735)	5.348 ± 0.072 (223,016)	5.061 ± 0.038 (115,145)
Pre Ag	10 mg/kg	222.3	2.809 ± 0.055 (645)[c]	2.784 ± 0.014 (607)	5.241 ± 0.046 (173,982)	5.062 ± 0.052 (115,342)
	50 mg/kg	237.0	3.098 ± 0.060 (1,253)	2.770 ± 0.134 (590)	5.475 ± 0.069 (298,393)	5.126 ± 0.143 (133,797)
	Solvent	179.2	3.243 ± 0.023 (1,752)	3.420 ± 0.021 (2,631)	5.496 ± 0.020 (312,979)	5.655 ± 0.029 (452,171)
Post Ag	10 mg/kg	184.2	3.340 ± 0.060 (2,186)	3.285 ± 0.040 (1,927)[c]	5.605 ± 0.056 (402,874)	5.555 ± 0.066 (358,749)
	50 mg/kg	191.2	3.269 ± 0.032 (1,856)	3.343 ± 0.077 (2,204)	5.550 ± 0.057 (354,700)	5.547 ± 0.101 (352,007)

[a]The drug was injected i.p. 1 day before or 1 day after 1×10^8 SRBC injected i.p. and PFC assays performed 5 days later.

[b]Log_{10} ± 1 s.e. from groups containing 10 mice for IgM and 5 mice for IgG. Numbers in parentheses are geometric means for each group. IgM data pooled from 2 experiments.

[c]Statistically significant reduction. ($p<0.01$ but >0.005 for IgM/10^6 on line 2 and $p<0.02$ but >0.01 for IgG/10^b on line 5).

TABLE 4

Effect of WR 180409 on anti-SRBC PFC response of mice[a]

Drug Treatment Time	Dose	Cells/Spleen x 10^{-6}	PFC per[b] 10^6 IgM	PFC per[b] 10^6 IgG	PFC per[b] Spleen IgM	PFC per[b] Spleen IgG
Pre Ag	Solvent	184.8	3.159 ± 0.055 (1,442)	3.533 ± 0.050 (3,411)	5.434 ± 0.069 (271,835)	5.833 ± 0.071 (680,741)
	10 mg/kg	204.7	3.060 ± 0.130 (1,148)	not tested	5.371 ± 0.143 (235,214)	not tested
	40 mg/kg	137.4[c]	3.089 ± 0.055 (1,227)	3.276 ± 0.078 (1,889)[c]	5.227 ± 0.069 (168,678)[c]	5.435 ± 0.122 (272,356)[c]
Post Ag	Solvent	218.3	3.194 ± 0.034 (1,564)	2.980 ± 0.044 (955)	5.537 ± 0.033 (344,511)	5.357 ± 0.095 (227,565)
	10 mg/kg	227.1	3.306 ± 0.042 (2,025)[d]	2.990 ± 0.124 (978)	5.663 ± 0.034 (459,913)[d]	5.345 ± 0.169 (221,562)
	40 mg/kg	171.9[c]	3.289 ± 0.031 (1,946)	3.116 ± 0.127 (1,306)	5.532 ± 0.057 (340,485)	5.317 ± 0.090 (207,425)

[a]The drug was injected i.p. 1 day before or 1 day after 1×10^8 SRBC injected i.p. and PFC assays performed 5 days later.

[b]Log_{10} ± 1 s.e. from groups containing 10 mice for IgM and 5 mice for IgG. Numbers in parentheses are geometric means for each group. IgM data pooled from 2 experiments.

[c]Statistically significant reduction. ($p<0.025$ but >0.01 for cells/spleen on line 3 and 6, and $p<0.05$ but >0.025 for PFC on line 3).

[d]Statistically significant enhancement ($p<0.02$ but >0.01).

TABLE 5

Effect of WR 171669 on the DH reaction of mice[a]

Drug Treatment Time	Dose	No. Mice/Group	DH Reaction[b] (relative ear reaction)
Pre Ag	Solvent	8	1.771 ± 0.279
	250 mg/kg	8	1.163 ± 0.061[c]
	1000 mg/kg	8	1.351 ± 0.142
Post Ag	Solvent	8	1.818 ± 0.282
	250 mg/kg	8	1.540 ± 0.176
	1000 mg/kg	8	1.394 ± 0.138

[a]The drug was injected i.p. 1 day before or 1 day after s.c. immunization with 5×10^6 SRBC. Mice were challenged intradermally 6 days later with 1×10^8 SRBC in the right ear and assayed the following day.

[b]Arithmetic mean of ratios for groups ± 1 s.e. calculated as follows:

$$\frac{\text{cpm in challenged ear}}{\text{cpm in control ear}}$$

[c]Statistically significant ($p < 0.025$ but > 0.02) suppression.

TABLE 6

Effect of WR 142490 on the DH reaction of mice[a]

Drug Treatment Time	Dose	No. Mice/Group	DH Reaction[b] (relative ear reaction)
Pre Ag	Solvent	8	1.846 ± 0.119
	10 mg/kg	7	1.755 ± 0.154
	40 mg/kg	7	1.490 ± 0.081[c]
Post Ag	Solvent	8	2.005 ± 0.336
	10 mg/kg	8	1.481 ± 0.175
	40 mg/kg	8	1.726 ± 0.114

[a]The drug was injected i.p. 1 day before or 1 day after s.c. immunization with 5×10^6 SRBC. Mice were challenged intradermally 6 days later with 1×10^8 SRBC in the right ear and assayed the following day.

[b]Arithmetic mean of ratios for groups ± 1 s.e. calculated as follows:

$$\frac{\text{cpm in challenged ear}}{\text{cpm in control ear}}$$

[c]Statistically significant ($p < 0.025$ but > 0.02) suppression.

TABLE 7

Effect of WR 172435 on the DH reaction of mice[a]

Drug Treatment Time	Dose	No. Mice/Group	DH Reaction[b] (relative ear reaction)
Pre Ag	Solvent	8	1.846 ± 0.119
	10 mg/kg	8	1.532 ± 0.146
	50 mg/kg	8	1.483 ± 0.148
Post Ag	Solvent	8	2.005 ± 0.336
	10 mg/kg	8	1.352 ± 0.208
	50 mg/kg	8	1.918 ± 0.217

[a]The drug was injected i.p. 1 day before or 1 day after s.c. immunization with 5×10^6 SRBC. Mice were challenged intradermally 6 days later with 1×10^8 SRBC in the right ear and assayed the following day.

[b]Arithmetic mean of ratios for groups ± 1 s.e. calculated as follows:

$$\frac{\text{cpm in challenged ear}}{\text{cpm in control ear}}$$

TABLE 8

Effect of WR 180409 on the DH reaction of mice[a]

Drug Treatment Time	Dose	No. Mice/Group	DH Reaction[b] (relative ear reaction)
Pre Ag	Solvent	8	1.867 ± 0.108
	10 mg/kg	8	2.056 ± 0.239
	40 mg/kg	7	1.688 ± 0.195
Post Ag	Solvent	8	1.919 ± 0.108
	10 mg/kg	8	1.787 ± 0.127
	40 mg/kg	5	2.370 ± 0.456

[a]The drug was injected i.p. 1 day before or 1 day after s.c. immunization with 5×10^6 SRBC. Mice were challenged intradermally 6 days later with 1×10^8 SRBC in the right ear and assayed the following day.

[b]Arithmetic mean of ratios for groups ± 1 s.e. calculated as follows:

$$\frac{\text{cpm in challenged ear}}{\text{cpm in control ear}}$$

TABLE 9

Effect of WR 171669 on the phagocytic function of mice[a]

Dose	Number Mice/Group	Phagocytic Index[b] 100 x K value	α value
Solvent	8	2.024 ± 0.139	3.591 ± 0.112
250 mg/kg	8	1.968 ± 0.245	3.396 ± 0.156
1000 mg/kg	8	1.099 ± 0.245[c]	2.974 ± 0.165[c]

[a]*The drug was injected* i.p. 2 days before test for phagocytic function.

[b]Arithmetic mean for groups ± 1 s.e.

[c]Statistically significant ($p < 0.001$ for K value and $p < 0.01$ but > 0.005 for α value) suppression.

TABLE 10

Effect of WR 142490 on the phagocytic function of mice[a]

Dose	Number Mice/Group	Phagocytic Index[b] 100 x K value	α value
Solvent	8	10.206 ± 0.913	5.929 ± 0.372
10 mg/kg	8	18.155 ± 0.672[c]	7.177 ± 0.245[c]
40 mg/kg	8	12.182 ± 1.125	5.354 ± 0.520

[a]The drug was injected i.p. 2 days before test for phagocytic functions.

[b]Arithmetic mean for groups ± 1 s.e.

[c]Significantly higher than controls ($p < 0.001$ for K and < 0.02 for α values).

TABLE 11

Effect of WR 172435 on the phagocytic function of mice[a]

Dose	Number Mice/Group	Phagocytic Index[b] 100 x K value	Phagocytic Index[b] α value
Solvent	8	10.206 ± 0.913	5.929 ± 0.372
10 mg/kg	8	15.661 ± 0.611[c]	6.182 ± 0.213
50 mg/kg	8	15.290 ± 1.057[c]	7.001 ± 0.183[c]

[a]The drug was injected i.p. 2 days before test for phagocytic functions.

[b]Arithmetic mean for groups ± 1 s.e.

[c]Significantly higher than controls ($p < 0.001$ for K value in group receiving 10 mg/kg, $p < 0.005$ for K value in group receiving 50 mg/kg, and $p < 0.025$ for α value in group receiving 50 mg/kg).

TABLE 12

Effect of WR 180409 on the phagocytic function of mice[a]

Dose	Number Mice/Group	Phagocytic Index[b] 100 x K value	α value
Solvent	8	7.522 ± 0.473	6.957 ± 0.182
10 mg/kg	8	10.343 ± 0.766[c]	6.802 ± 0.137
40 mg/kg	8	6.848 ± 1.196	6.938 ± 0.271

[a]The drug was injected i.p. 2 days before test for phagocytic functions.

[b]Arithmetic mean for groups ± 1 s.e.

[c]Significantly higher than controls. ($p < 0.01$).

TABLE 13

Splenic and hepatic changes in mice injected with WR 142490[a]

Dose	No. Mice/Group	Weight Index[b,d] Spleen	Weight Index[b,d] Liver	^{51}Cr-SRBC Uptake[c] Spleen	^{51}Cr-SRBC Uptake[c] Liver
Solvent	8	6.836 ± 0.371	73.200 ± 3.997	244,026 ± 19,059	435,470 ± 24,790
10 mg/kg	8	6.145 ± 0.146	73.180 ± 2.234	171,103 ± 13,866[e]	430,933 ± 21,006
40 mg/kg	8	6.165 ± 0.220	79.427 ± 2.294	194,762 ± 25,761	381,015 ± 22,704

[a]The drug was injected i.p. 2 days before the i.v. injection of 5×10^8 ^{51}Cr-SRBC. Mice were killed 20 min after SRBC injection and spleens and livers were excised, weighed and assayed for radioactivity.

[b]Spleen or liver weight/whole body weight x 1000.

[c]Counts per minute/organ weight.

[d]Arithmetic mean ± 1 s.e.

[e]Statistically significant reduction.

TABLE 14

Splenic and hepatic changes in mice injected with WR 172435[a]

Dose	No. Mice/Group	Weight Index[b,d] Spleen	Weight Index[b,d] Liver	^{51}Cr-SRBC Uptake[c] Spleen	^{51}Cr-SRBC Uptake[c] Liver
Solvent	8	6.836 ± 0.371	73.200 ± 3.997	244,026 ± 19,059	435,470 ± 24,790
10 mg/kg	8	7.299 ± 0.244	80.280 ± 2.069	199,480 ± 9,586	442,388 ± 13,326
40 mg/kg	8	6.642 ± 0.348	71.201 ± 2.129	195,534 ± 13,297	449,300 ± 15,498

[a]The drug was injected i.p. 2 days before the i.v. injection of 5×10^8 ^{51}Cr-SRBC. Mice were killed 20 min after SRBC injection and spleens and livers were excised, weighed and assayed for radioactivity.

[b]Spleen or liver weight/whole body weight x 1000.

[c]Counts per minute/organ weight.

[d]Arithmetic mean ± 1 s.e.

TABLE 15

Splenic and hepatic changes in mice injected with WR 180409[a]

Dose	No. Mice/Group	Weight Index[b,d] Spleen	Weight Index[b,d] Liver	^{51}Cr-SRBC Uptake[c] Spleen	^{51}Cr-SRBC Uptake[c] Liver
Solvent	8	4.607 ± 0.086	56.096 ± 1.781	267,633 ± 21,352	200,890 ± 7,757
10 mg/kg	8	5.706 ± 0.243	62.392 ± 1.730	165,639 ± 9,226[e]	232,190 ± 15,495
40 mg/kg	8	3.897 ± 0.447	53.546 ± 1.526	200,864 ± 41,989	231,308 ± 12,530

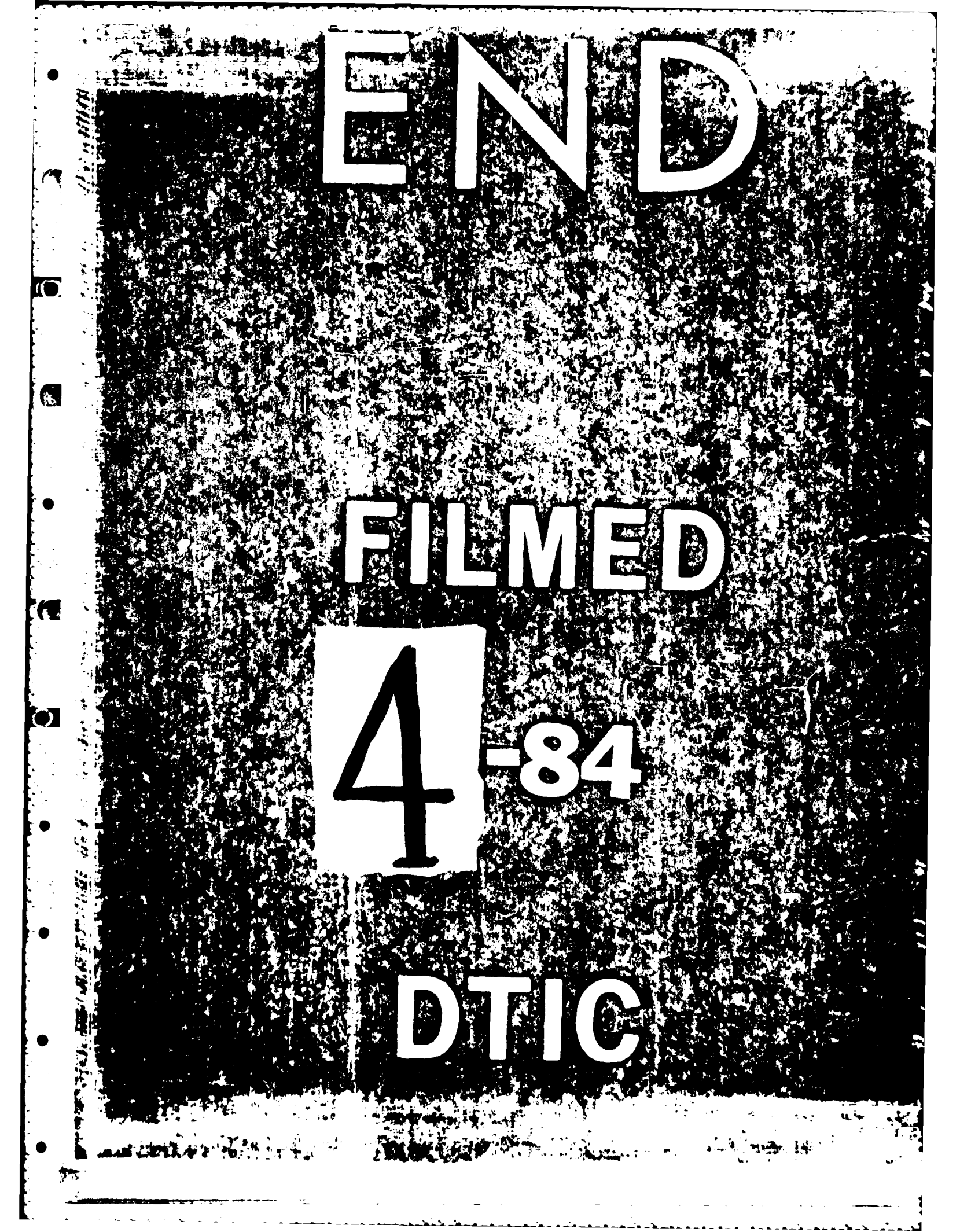
[a]The drug was injected i.p. 2 days before the i.v. injection of 5×10^8 ^{51}Cr-SRBC. Mice were killed 20 min after SRBC injection and spleens and livers were excised, weighed and assayed for radioactivity.

[b]Spleen or liver weight/whole body weight x 1000.

[c]Counts per minute/organ weight.

[d]Arithmetic mean ± 1 s.e.

[e]Statistically significant reduction.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